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Nature & Science For Better Health

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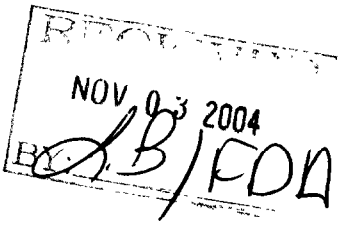
October 27, 2004



Division of Dietary Supplement Programs
Office of Nutritional Products, Labeling, and Dietary Supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park MD 20740-3835

RE: New Dietary Ingredient Notification Pursuant to 21 CFR §190.6

Dear Sir or Madam:

American BioSciences, Inc., submits the enclosed information to the Food and Drug Administration as required by Section 413(a) of the Federal Food Drug and Cosmetic Act in anticipation of its marketing of a dietary supplement which contains the new dietary ingredient Avemar. American BioSciences intends to incorporate the Avemar ingredient into a dietary supplement.

As required, we enclose an original and two copies of the notification and its attachments.

Since this submission is made under section 413 of the Act, we request that it be accorded the 90-day confidentiality provisions relating to public notice. Portions of the enclosed document that are regarded as confidential commercial information are clearly indicated, and we request that this information not be disclosed.

If you have any questions, please do not hesitate to call me at 845-727-0800.

Sincerely,
American BioSciences, Inc.

David Wales
President

Encl.

NEW DIETARY INGREDIENT NOTIFICATION

AVEMAR

Submitted to:

U.S. FOOD AND DRUG ADMINISTRATION
CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
OFFICE OF NUTRITIONAL PRODUCTS, LABELING,
AND DIETARY SUPPLEMENTS
DIVISION OF DIETARY SUPPLEMENT PROGRAMS

Submitted by:

AMERICAN BIOSCIENCES, INC.

NOVEMBER, 2004

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1. Name and Mailing Address of the Distributor

American Biosciences, Inc.
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E-mail:	dwales@americanbiosciences.com

2. Name of the New Dietary Ingredient

The trade name of the new dietary ingredient is Avemar. It is also known as MSC, fermented wheat germ, and fermented wheat germ extract. In the production of the product, maltodextrin is mixed with the fermented wheat germ extract prior to spray drying to form a powder. Colloidal silicon dioxide is added to prevent adherence of the mixture onto the wall of the dryer. The name Avemar is often used to refer to the ready-to-use form of the product in which fructose, flavoring, and other ingredients are added to the fermented dried wheat germ powder. This notification, however, regards only the fermented dried wheat germ extract itself. Since the product is an extract of yeast fermented wheat germ, it has long been consumed by Americans as part of their diets and is thus a "dietary ingredient" as defined in the Dietary Supplement Health and Education Act.

3. Description

A. Level of the NDI in the Supplement

5.3 g wheat germ extract in 9 g of the dietary supplement (59 percent w/w).

B. Conditions of Use in the Labeling (Amount, Limitations)

The 9-g dose of the supplement, containing 5.3 g of the NDI, fermented dried wheat germ, is to be taken once per day, with the Avemar microgranules dissolved in 150 mL water. It is recommended that the dietary supplement be taken within 1 hour before or after meals. For a 70-kg adult, this represents a daily exposure of 75.7 mg/kg of fermented dried wheat germ.

The label will recommend that Avemar not be taken by children or by women who are pregnant or breast feeding. It should not be taken by those who have undertaken organ or tissue transplants, or those who suffer from bleeding erosions or bleeding ulcers of the gastrointestinal tract, enteritis, colitis, or malabsorption syndrome. Patients taking prescription medications should consult with their doctor before use.

The label will also state prominently that the product contains gluten.

4. History of Use or Other Evidence of Safety

A. Product Characterization

(1) Manufacturer

Avemar is manufactured by Biropharma First Hungarian Biotechnological Ltd., a subsidiary of Biromedicina Corporation. Manufacturing facilities are located at 6413 Kunfeherto, District IV, No. 6, Hungary. Quality assurance systems, including NACCP and GMP, have been assessed and registered as meeting the ISO 9001:2000 standard and the standards for GMP production of pharmaceuticals by the National Institute of Pharmacy, Ministry of Health, Hungary. Certificates of these qualifications are attached as Appendix 2.

(2) Product Specifications

The fermented wheat germ is standardized to contain approximately 325±55 μg/g 2,6-dimethoxy-p-benzoquinone (2,6-DMBQ). The fermented dried wheat germ powder comprises 63.2 percent fermented wheat germ extract, 35.0 percent maltodextrin, and 1.8 percent colloidal silicon dioxide. Its 2,6-DMBQ content is approximately 205±35 μg/g. It meets the specifications shown in Table 1.

Table 1. Specifications for Fermented Dried Wheat Germ Powder (Avemar)

Parameter	Specification	Test
Appearance	Light gray-brown microgranules	Inspection
Odor and taste	Yeast-like; sourish; bitter aftertaste	Inspection
Particle size distribution	NMT* 8% > 0.8 mm NMT 25% < 0.16 mm	Screening; Ph.Hg.VII.I/ B.1.3.1.
Moisture	NMT 2.6%	Infrared analysis
Density	34-40 g/100 cm^3 ± 15%	Ph.Eur 4.2
pH	4.0 – 5.2	Ph.Eur 4.1
Ash	NMT 10.5%	Ph.Eur 4.2
2,6-DMBQ assay	NLT* 170 µg/g NMT 240 µg/g	HPLC
Total aerobic bacteria	NMT 10^3/g	Ph.Eur 4.1
Total aerobic fungi	NMT 10^2/g	Ph.Eur 4.1
E. coli	0 in 1 g	Ph.Eur 4.1
Staphylococcus aureus	<10/g	Ph.Eur 4.1
Enterobacteriaceae	<10/g	Ph.Eur 4.1
Bacillus cereus	<10/g	Ph.Eur 4.1
Enterococcus fecalis	<10/g	Ph.Eur 4.1
Salmonella	0 in 25 g	Ph.Eur 4.1
Lead	NMT 0.3 µg/g	ICP-AES
Arsenic	NMT 0.3 µg/g	ICP-AES
Mercury	NMT 0.3 µg/g	ICP-AES
* NMT = not more than; NLT = not less than		

(3) Product Stability

A sample from one lot of ready-to-use Avemar was stored at 17-25°C and 55-70% relative humidity in its normal plastic container for a period of 2 years. Samples were taken for testing every 3 months. In Table 2 are shown the results of the testing at the start, 6 months, 12 months, 18 months, and 24 months. As is evident, there were no significant changes in any of the tested parameters. Avemar is thus regarded as having been shown to be stable, under proper storage conditions, for at least 2 years.

Table 2. Stability Over 24 Months of Ready-to-Use Avemar

Parameter	Start	6 mo	12 mo	18 mo	24 mo
Color	Light brown	No change	No change	No change	No change
Odor	Yeasty	No change	No change	No change	No change
Taste	Yeasty, sour, bitter	No change	No change	No change	No change
Density (g/100 cm^3)	34.2	34.3	34.5	34.5	34.7
Granularity	Free-flowing, microgranulate	No change	No change	No change	No change
pH	4.74	4.73	4.75	4.71	4.71
Moisture (%)	2.95	2.98	2.99	3.00	2.98
2,6-DMBQ (µg/g)	163	169	162	173	170
Total plate count	7 log 2	6 log 2	1 log 3	9 log 2	1.2 log 3
Mold count	<10	<10	<10	<10	<10
E. coli	0	0	0	0	0

B. Raw Ingredients

(1) Wheat Germ

Supplier: Diamant Mill International, Hungary (NACCP and ISO-9001 qualified)

Definition: Wheat (*Triticum vulgare*) germ separated from wheat during grinding, intended to be used for human consumption

The wheat germ complies with National Standards of Hungary 08 1361-80: Wheat Germ for Human Consumption, except with regard to moisture content. These specifications are shown below in Table 3. The supplier certifies that no genetically modified organisms (GMOs) are used in the production of the wheat germ and that pesticide residues, heavy metals, and other chemical environmental contaminants are all within the limits permitted in foods by Health Ministerial Decree 17/1999 (VI.16), which has been harmonized with EU directives.

Table 3. Specifications for Wheat Germ for Human Consumption

Parameter	Specification*	Test
Description	Yellowish platelets 0.8-2 mm diameter and 0.1-0.25 mm thickness	Inspection
Organoleptic	Not rancid, musty, moldy, decayed, burned, or clotted	Inspection
Bran (% w/w)	NMT 22	Inspection
Insect fragments	None	Inspection
Grit (% w/w)	NMT 0.05	MSZ 6369/2-85
Particle fraction < 0.5 mm (%)	NMT 15	Sieving analysis
Microbiology		Ph. Eur 4
Total plate count (count/g)	NMT 10^4	
Mold (count/g)	NMT 10^2	
Enterobacteriaceae (count/g)	NMT 10^2	
E. coli (1.0 g)	0	
Salmonella (10.0 g)	0	
Mycotoxins (µg/kg)		HPLC OMMI method
Total aflatoxin (B1+B2+G1+G2)	NMT 4	
Deoxynivalenole	NMT 1000	
Zearalenone	NMT 100	
T2 toxin	NMT 300	
Acid value (mg KOH/g)	NMT10.0	MSZ 3633-81
Moisture content (%)	NMT 15.0	Loss on drying
Crude ash (% DM)	NMT 5.0	GT 41137/87
Crude fat (% DM)	NLT 8.0	MSZ 6367/14
Crude protein (% DM)	NMT 26	MSZ 6367/11
* NMT = not more than; NLT = not less than		

Wheat germ is stored at 5-25°C for a maximum of 3 months prior to being used to produce Avemar.

(2) Baker's Yeast (Saccharomices cerevisiae)

Supplier: Budafok Eleszto-Es Szeszgyar Kft, a Hungarian subsidiary of the Lasaffre Group.

From the type collection at the Pasteur Institute of Paris, CNCM I-2970 (Collection Nationale de Cultures de Microorganismes).

Baker's yeast is stored at 5-7°C for a maximum of 1 month prior to use.

C. Manufacturing Method

A fixed amount of food-grade wheat germ and water are placed in stainless steel fermentation vessels and a fixed amount of baker's yeast is introduced. (The ratio of wheat germ to yeast is approximately 3:1, and the ratio of water to dry matter is approximately 9:1.) Fermentation proceeds with continuous stirring at controlled pH, temperature, and flow of filtered air for a fixed period of time, approximately 18 hours. The fermentation product is decanted, separated, and fine-filtered to a cell-free solution, which is condensed under vacuum to a specified weight percentage. Food-grade maltodextrin and colloid silicon dioxide are added and the mixture is spray-dried. Finally, the product is microgranulated and packed.

D. Evidence of Safety

A large amount of evidence is available to demonstrate the safety of Avemar under its intended conditions of use. Most obviously, the product is an extract of wheat germ fermented with the *Saccharomices cerevisiae* strain of yeast, both of which have long histories of safe use in the food supply. Additionally, Avemar has been investigated in numerous animal and human studies of its efficacy; in none of these studies has any indication of adverse effects been identified. Avemar has been sold in numerous countries for a number of years with no reports of adverse effects. Finally, Avemar has been subjected to acute toxicity studies in the rat and in the mouse, a subacute toxicity study in the rat, and subchronic toxicity studies in the rat and in the mouse, in addition to genotoxicity screening tests, and has been evaluated for hematologic effects in multi-year studies in human cancer patients.

(1) Wheat Germ

Wheat germ is a food: the germ is part of the wheat kernel, along with the bran and the endosperm. Like the bran, the germ is removed in the processing of wheat to yield white flour, but it is retained in whole wheat products. *Healthy People 2010* (DHHS 2000) recommends that Americans consume at least 6 servings of grain products daily, with at least 3 being whole grains. In comparison, Cleveland et al. (2000), using data from the 1994-96 Continuing Survey of Food Intakes by Individuals, estimated that American adults consume an average of 6.7 servings of grains daily, but only about 1.0 of these were whole grains.

The germ constitutes approximately 2.5 percent of the total weight of the wheat kernel. Since a loaf of whole-wheat bread contains about 2 cups of whole-wheat flour, or about 270 g, it includes about 7 g wheat germ. Additionally, wheat germ is used as an ingredient in a wide variety of foods formulated to provide advantageous nutritional characteristics. While it is likely that the average American's current consumption of wheat germ is <1 g/day, it appears probable that many Americans—those with a preference for whole grain foods—may consume >5 g/day of wheat germ.

(2) Baker's Yeast

Saccharomices cerevisiae, also known as baker's yeast, has been used in the production of fermented bakery and brewing products since ancient times. It is more widely used in food production than any other yeast species (Dyer 2002). It is one of the most fundamental and studied model organisms in molecular and cell biology. Indeed, *S. cerevisiae* was the first eukaryote to have its complete genome sequenced, published in 1996 (Goffeau et al. 1997); the genome database, funded by the National Institutes of Health, is available at http://www.yeastgenome.org.

Baker's yeast appears at several places in FDA's "Partial List of Microorganisms and Microbial-Derived Ingredients That Are Used in Foods," a list last updated in July 2001 and available on FDA's website at http://vm.cfsan.fda.gov/~dms/opa-micr.html.

Although the yeast are killed and their cells disrupted in the production of Avemar, fragments and molecular constituents of the yeast cells are found in the final product. Baker's yeast protein and glycan, as well as dried Baker's yeast, are all permitted for food use at 21 CFR §172.325, §172.898, and §172.896, respectively.

(3) Use in Other Countries

Avemar was first introduced as a dietary supplement in Hungary in the autumn of 1998. In February, 2002, it was approved and registered as a medical nutriment for cancer patients by the National Institute of Food Hygiene and Nutrition of the National Center for Public Health, Hungary (registration number 503). Avemar is registered and sold over-the-counter as a "medical nutriment" for cancer patients in two countries in eastern Europe in addition to Hungary: the Czech Republic (registration no. HEM-3512-13.103-1178) and Bulgaria (registration no. 05180/2003). It is also sold in Italy, Switzerland, Cyprus, Russia, Israel, Austria, Slovakia, South Korea, Taiwan, Japan, Hong Kong, Australia, and New Zealand. In Australia Avemar is registered by the Therapeutic Goods Administration of Australia as an immunomodulant therapeutic product (registration no. AUST L 95037).

(4) Animal Studies

a. Non-Toxicology Studies

The beneficial effects of Avemar have been investigated in a number of published and unpublished studies using mice and rats as models. While the potential benefits of Avemar are not relevant to this discussion of safety, these studies also provided the opportunity to observe any adverse effects of Avemar on the experimental animals. This is of some interest, because the dosing levels were occasionally quite high, and the study periods were sometimes much longer than is usually true of toxicology studies. Note that the test article in these studies was usually the ready-to-use form of Avemar, containing approximately 59 percent fermented dried wheat germ.

Four non-toxicology animal studies (3 of them published: Hidvegi et al. 1998; Hidvegi et al. 1999; Szende et al. 2004; and 1 unpublished: Telekes et al. undated) are summarized in Table A1.1 in Appendix 1. In 3 studies the Avemar dose was 3000 mg/kg/day, equivalent to 210 g/day for a 70-pound human. In one study (Telekes et al. undated), a range of doses as high as 5000 mg/kg/day was used. The duration of administration of Avemar was from 15 days (Telekes et al. undated) to 65 days (Hidvegi et al. 1998).

In no case were there any Avemar treatment-related deaths among the experimental animals, and no adverse reactions were reported. One study (Zalatnai et al. 2001) compared the weights of animals receiving Avemar with those that did not and found no difference; this study also reported no macroscopically visible intestinal lesions in rats receiving Avemar alone.

b. Toxicology Studies

(i) Acute Oral Toxicity Study in the Mouse

A study of the acute oral toxicity of Avemar in the mouse was conducted in 1999 by the Department of Pharmacology and Toxicology of the University of Veterinary Science in Budapest, Hungary (University of Veterinary Science 1999a). This study was performed according to the Good Laboratory Practice (GLP) guidelines provided in regulation no. P-44-1992 of the Hungarian National Institute of Pharmacy and complying with GLP for Testing of Chemicals, ENV/MC/CHEM (98)17. The study was performed with limit-testing according to Guidelines for the Testing of Chemicals (no. 401) promulgated by the Organization for Economic Cooperation and Development (OECD).

Avemar was dissolved in distilled water at a concentration of 8 g Avemar per 100 mL distilled water and given orally via gavage to CD-1 mice provided by Charles River Hungary Ltd. The mice were 5 weeks old at study initiation; males weighed 22.4-27.4 g, females weighed 19.6-23.7 g. After 1 week of observation, 10 mice of each sex were randomly assigned as test animals and 10 mice of each sex as controls. The animals

were caged 5 animals per cage, fed ad libitum with Charles River VRF1 autoclaved mouse and rat diet, and allowed ad libitum access to tap water.

Since the test article was regarded as nontoxic, the dose was set at the limit of 2000 mg/kg and was given in a single administration; control animals received an equal volume (about 0.5 mL) of distilled water. The animals were observed twice a day for 14 days post-treatment; observations included the state of the skin, fur, eyes and mucous membranes; respiratory function; circulation; autonomic nervous function; somatomotor activity; trembling; salivation; diarrhea; and general behavior. Postmortem examinations were performed after sacrifice.

No animal deaths occurred in either the control or test group, and no abnormal clinical signs were observed in either group during the post-treatment observation period. There were no significant differences between the two groups in the weights of animals of either sex at 24 hours, 7 days, or 14 days. The pathological examination revealed no macroscopic lesions in the animals of either group.

Based on the absence of adverse effects, the oral LD_{50} of Avemar in male and female mice is > 2000 mg/kg. This is equal to 1178 mg/kg of fermented dried wheat germ.

(ii) Acute Oral Toxicity Study in the Rat

A study of the acute oral toxicity of Avemar in the rat was conducted in 1999 by the Department of Pharmacology and Toxicology of the University of Veterinary Science in Budapest, Hungary (University of Veterinary Science 1999b). This study was performed according to the GLP guidelines provided in regulation no. P-44-1992 of the Hungarian National Institute of Pharmacy and complying with GLP for Testing of Chemicals, ENV/MC/CHEM (98)17. The study was performed with limit-testing according to Guidelines for the Testing of Chemicals (no. 401) promulgated by OECD.

Avemar was dissolved in distilled water at a concentration of 20 g Avemar per 100 mL distilled water and given orally via gavage to Wistar BR rats provided by Charles River Hungary Ltd. The rats were 5 weeks old at study initiation; males weighed 87.25-111.34 g, females weighed 80.40-112.60 g. After 1 week of observation, 10 rats of each sex were randomly assigned as test animals and 10 rats of each sex as controls. The animals were caged 5 animals per cage, fed ad libitum with Altromin GmbH rat chow, and allowed ad libitum access to tap water.

Since the test article was regarded as nontoxic, the dose was set at the limit of 2000 mg/kg and was given in a single administration; control animals received an equal volume (about 1.0 mL) of distilled water. The animals were observed twice a day for 14 days post-treatment; observations included the state of the skin, fur, eyes and mucous membranes; respiratory function; circulation; autonomic nervous function; somatomotor

activity; trembling; salivation; diarrhea; and general behavior. Postmortem examinations were performed after sacrifice.

No animal deaths occurred in either the control or test group, and no abnormal clinical signs were observed in either group during the post-treatment observation period. There were no significant differences between the two groups in the weights of animals of either sex at 24 hours, 7 days, or 14 days. The pathological examination revealed no macroscopic lesions in the animals of either group.

Based on the absence of adverse effects, the oral LD_{50} of Avemar in male and female rats in this study is > 2000 mg/kg. This is equal to 1178 mg/kg of fermented dried wheat germ.

A similar acute toxicology study was conducted with Wistar rats at the Institute of Preventive and Clinical Medicine, Ministry of Health, Slovakia. In the Slovakian study, the limit dose was set at 5000 mg/kg. Avemar, dissolved in distilled water, was administered via gavage. There was no difference between the test animals and controls in the weights of the animals, and no cardiovascular, respiratory, neurological, or other toxic effects were observed. Based on the absence of adverse effects, the oral LD_{50} of Avemar in male and female rats in this study is > 5000 mg/kg = 2944 mg/kg fermented dried wheat germ.

(iii) Subacute Oral Toxicity Study in the Rat

A study of the subacute oral toxicity of Avemar in the rat was conducted in 2000 by the Department of Pharmacology and Toxicology of the University of Veterinary Science in Budapest, Hungary (University of Veterinary Science 2000). The histopathological examination was carried out at the Histopathological Department of the Central Veterinary Institute, Budapest. The study did not meet OECD specifications for a subacute study in that it had only a single dose level rather than the three recommended, but was conducted under Good Laboratory Practice (GLP) and otherwise in accordance with OECD guidelines.

Avemar was dissolved in distilled water at a concentration of 20 g Avemar per 100 mL water and given orally via gavage to Wistar BR rats provided by Charles River Hungary Ltd. The rats were 4.5 weeks of age at the beginning of the study. After a 6-day period of observation, 20 rats of each sex were randomly assigned as test animals and 20 rats of each sex as controls. The animals were caged 5 animals per cage, fed *ad libitum*, and allowed *ad libitum* access to tap water.

Since the test article was regarded as nontoxic, the dose was set at the limit of 2000 mg/kg/day and was given in a single daily administration; control animals received an equal volume (about 10 mL/kg) of a 1% methylcellulose suspension alone daily. Both the test and control substances were analyzed for precision of concentration and for stability; results of both analyses were within requirements.

Administration continued over a period of 28 days. Half of the animals (10 of each sex from both the test and control groups) were sacrificed immediately, while the remaining animals were observed for 14 days post-treatment and then sacrificed. Prior to sacrifice, all animals were starved and blood samples were taken from the femoral vein for study of hematology and clinical chemistry; urine samples were taken for urinalysis.

Animals were observed twice daily during both the test and follow-up period and observations recorded for feed consumption, water intake, mortality, clinical signs, skin, fur, eyes, mucosae, respiration, circulation, autonomic nervous system, somatomotor activity, trembling, diarrhea, salivation, and overall behavior. Postmortem examinations were performed after sacrifice. The following organs were weighed: liver, heart, spleen, thymus, kidneys (right and left), testes (right and left), epididymes, ovaries (right and left). Organs fixed and examined for the histopathological analysis were: lymph nodes, mammary glands, salivary glands, sternebrae, femur, vertebrae, bone marrow, hypophysis, thymus, trachea, lungs, heart, thyroid, esophagus, stomach, intestine, colon, liver, gallbladder, pancreas, spleen, kidneys, adrenals, bladder, prostate, testes, ovaries, uterus, brain (three coronal sections), eyes, and spinal chord.

No significant test-article related differences were found between the test and control animals in body weight, clinical symptoms, feed consumption, water intake, clinico-chemical analyses, urinalysis, pathological examinations, organ weights, or histopathology. The hematological analysis found slightly increased hemoglobin content of female rats in the test group; this difference was judged to be of no biological importance and not caused by the test substance. During the 28-day treatment period 3 rats died, all in the test group, but the results of the pathological examination indicated that the deaths were not due to any toxic effect of the test substance. No deaths occurred during the 14-day post-treatment observation period.

Based on the absence of adverse effects, the no observable adverse effect level (NOAEL) of Avemar in this study was determined to be the tested dose of 2000 mg/kg/day. This is equal to 1178 mg/kg/day of fermented dried wheat germ.

(iv) Subchronic Oral Toxicity Study in the Rat and in the Mouse

A 77-day toxicity study was performed with F344 rats and C57BL/6 mice (First Institute of Pathology 1997). The animals were treated daily with a dose of 3000 mg/kg Avemar (diluted in 5 mL water) via gavage. There were 13 male rats and 13 male mice in the two test groups and equal numbers in the control. The animals were observed daily for pathological signs (fur, color of nose and ears, body temperature) and were weighed daily. After sacrifice via anesthesia on day 77, samples were taken from organs and tissues for histological examination.

There were no differences in the weight gains of the test and control animals and no histopathological alterations were found in either test or control animals. The no

observable adverse effect level (NOAEL) of Avemar in this study was determined to be the tested dose of 3000 mg/kg/day. This is equal to 1767 mg/kg/day of fermented dried wheat germ.

(v) Evaluation of the Mutagenicity of Avemar in Drosophila

In one series of tests, the mutagenicity in *Drosophila melanogaster* was evaluated for nickel chloride, cobalt chloride, urethane, and formaldehyde, alone or in combination with Avemar (National Institute of Chemical Safety 2004). In all cases the test materials containing Avemar had lower mutagenicity than did the same compounds without Avemar. In another experiment, the mutagenicity of Avemar was compared with living yeast as a positive control and standard nutrient as a negative control; Avemar appeared to be less mutagenic than either the positive or negative control. A final test compared the mutagenicity of Avemar with sacharose, and again found no evidence of mutagenic effects from Avemar.

(vi) Evaluation of the Genotoxicity of Avemar (Rat Micronucleus Test)

For this study (Zalatnai et al. 2001), 100 4-week-old inbred male F344 rats were randomized into 3 test groups and an n=10 untreated control group (group 1). In group 2 (n=48), animals received no Avemar but were given subcutaneous injections 1 week apart of 15 mg/kg of azoxymethane (AOM) dissolved in physiologic saline. In group 3 (n=32), animals were given 3000 mg/kg Avemar via gavage once a day for 2 weeks prior to the first injection of AOM and throughout the remaining period of the study. In group 4 (n=10), animals were administered Avemar but were not injected with AOM. All animals were killed by exsanguination after 32 weeks and the marrow removed from a femur.

Determination of the frequencies of micronucleided polychromasia erythrocytes and of immature erythrocytes indicated that, within the experimental conditions, Avemar is not genotoxic.

(5) Human Clinical Trials

a. Efficacy Studies

The beneficial effects of Avemar have been investigated in a number of published and unpublished human clinical trials. As in the animal studies, the test article in these studies was ready-to-use Avemar, containing approximately 59 percent (w/w) fermented dried wheat germ. While the potential benefits of Avemar are not relevant to this discussion of safety, these studies also provided the opportunity to observe any adverse effects of Avemar on the participants in the trials. Many of the study participants were in late stages of terminal cancer or otherwise in weakened states during which they might be

expected to be unusually sensitive to any toxicity of the compound. Eight such studies are summarized in Table A1.2 in Appendix 1.

One study (Jakab et al. 2003) has appeared in the published literature. In this open-label multicenter cohort study, postsurgical colorectal cancer patients received standard therapy. Based on patient choice, 66 patients also received 9 g/day of Avemar, while 104 control patients did not. Those receiving Avemar continued this treatment for a period of 6 months or longer. No serious events (defined as NCI-CTC grades 3-4) were observed. Diarrhea occurred in 4 patients receiving Avemar, nausea in 2, and flatulence, repletion, soft stools, and constipation each in 1. In general, there were no more side effects in those receiving Avemar than those not receiving it.

Three unpublished studies were available only in the form of abstracts (Ajkay et al. 2000; Balogh et al. 2001a, 2001b). In these studies, unreported doses of Avemar were given as adjunct therapy to 17, 39, and 55 patients suffering from terminal lung cancer (Ajkay et al. 2000) or breast cancer (Balogh et al. 2001a, 2001b). The dosing periods averaged 7.9, 19.3, and 32.2 months, respectively. In none of these studies with patients in extremely poor health were any adverse side effects noted from the Avemar treatment.

In another currently unpublished study, Ujpal et al. (undated) gave 9 g/day of Avemar to 22 postsurgical oral cancer patients for 12 months. Compared with 21 patients serving as historical controls, no indications of adverse side effects were reported. In a similar unpublished study with postsurgical patients with skin melanomas receiving treatment with dacarbazine, Demidov et al. (2002) randomly assigned 22 patients (13 M, 9 F) to receive 9 g/day of Avemar while 24 patients (15 M, 9 F) served as controls. After 12 months, the patients receiving Avemar had fewer reports of all adverse events (nausea, fatigue, fever, leukopenia, thrombocytopenia, diarrhea) than did the controls; no adverse effects were observed in the test group.

In a yet unpublished study of pediatric patients (average age = 11 years) with proven solid malignant tumors, Garami et al. (undated) assigned patients to 11 matched pairs and then randomly assigned one of each pair to receive 12 g/m^2/day of Avemar. This dose is estimated to have been about 6.25 g/day, or 192 mg/kg/day. Patients remained in the study for an average of 29 months; no serious events (NCI-CTC grades 3-4) were observed.

Finally, Telekes et al. (undated) carried out an unpublished feasibility study to investigate the value of Avemar in the treatment of rheumatoid arthritis. In this open-label study, 15 patients age 44-68 years received 18 g/day of Avemar in 2 daily doses of 9 g each for 12 months. Side effects were evaluated at baseline and at 6 and 12 months. No adverse side effects were observed and no patient withdrew from the study.

In all, 247 patients in poor health received doses of Avemar averaging about 9 g/day for periods ranging from 6 to more than 32 months, with no adverse reactions observed.

b. Study of Hematologic Effect of Avemar

The effect of long-lasting administration of Avemar on the hematologic status of carcinoma patients was examined in two hospital centers (Necropsy undated). In one center, the hematologic status of 25 mammary carcinoma patients (group Ma) and 23 colorectal carcinoma patients (group Ca) was assessed prior to initiation of treatment and at 6 months and 1 year. In the second center, the hematologic status of 25 mammary carcinoma patients (group Mb) and 25 colorectal carcinoma patients (group Cb) was assessed prior to initiation of treatment, at 1 year, and at 3 years.

Hematologic data included white blood cell count, red blood cell count, hemoglobin level, hematocrit, platelet count, erythrocyte sedimentation rate, lymphocyte count, neutrophil granulocyte count, monocyte count, eosinophil granulocyte count, and prothrombin level. Several changes were observed (increased prothrombin in group Ma; increased monocytes and erythrocyte sedimentation and decreased hemoglobin and hematocrit in group Mb; increased monocytes and decreased platelets, neutrophil granulocytes, and erythrocyte sedimentation in group Cb), but all values remained within normal limits and were judged to be of no biological significance.

(6) Conclusion

Avemar is a well characterized substance produced using starting materials that have a long history of safe use in the U.S. diet. The starting materials are food-grade, and Avemar is produced under carefully controlled conditions that ensure its purity and lot-to-lot consistency. It has been administered to experimental animals at high doses for extended periods of time without any evidence of toxicity, and has been administered to humans—including humans in late stages of cancer with weakened resistance—without adverse effects. It has been marketed over the counter in numerous countries for several years without any indication of adverse effects. The safety of the product has been evaluated in acute toxicity, subacute toxicity, subchronic toxicity, and genotoxicity studies, which have found it to be non-toxic at exposures far in excess of those that are expected to result from its intended use.

We conclude that adequate evidence exists that demonstrates with reasonable assurance that Avemar, under its intended conditions of use, meets the safety standard expressed in the Dietary Supplement Health and Education Act of 1994 and does not pose a significant or unreasonable risk of illness or injury.

5. References

NOTE: COPIES OF STUDIES MARKED * ARE INCLUDED IN APPENDIX 3

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APPENDIX 1

Summaries of Animal and Human Studies of Avemar

Table A1.1. Non-Toxicology Studies in Animals: Findings Regarding Safety						
Citation	**Objective**	**Study Design**	**Species**	**Dose**	**Duration**	**Safety Results**
Hidvegi et al. 1998	Study the effect of Avemar and Avemar+Vitamin C on tumor growth and metastasis.	Tumor cells were injected into the spleens of test animals. Animals were given Avemar, Avemar+Vitamin C, or water via gavage.	8-10-week old inbred rats, 8-10-week old inbred mice; n's were not reported.	3000 mg/kg/d in a single dose	35, 45, and 65 days	No adverse reactions or treatment deaths were reported.
Hidvegi et al. 1999	Investigate the effect of Avemar on blastic transformation of peripheral blood lymphocytes.	Spleen cells were harvested from treated v. control mice and exposed to Concavavalin A to cause blastic transformation.	8-week-old C_{57}BL male and female mice; 20 given 3000 mg/kg/d Avemar, 50 controls.	3000 mg/kg/d, 5 days/week	6 weeks	No adverse reactions or treatment deaths were reported.

Table A1.1. Non-Toxicology Studies in Animals: Findings Regarding Safety						
Citation	**Objective**	**Study Design**	**Species**	**Dose**	**Duration**	**Safety Results**
Szende et al. 2004	Study the effects of simultaneous administration of Avemar and cytostatic drugs.	Animals were inoculated with a highly metastatic line of Lewis lung carcinoma cells (3LL-HH), then treated with various combinations of Avemar and cytostatic drugs.	8-10-week-old C_{57}BL male and female mice; 35 animals received a cytostatic drug+Avemar, 30 received Avemar alone, 35 received a drug alone, and 35 were controls.	3000 mg/kg/d via gavage in a single dose	Until death from tumor inoculation, 18-60 days	All animal deaths were due to the inoculated tumors and the addition of Avemar to cytostatic drugs had no adverse effect on tumor growth or time to death.
Telekes et al. (unpubl and undated)	Investigate the effect of Avemar v. standard drugs on the rat adjuvant arthritis model for human rheumatoid arthritis.	Rats were injected in one paw with Mycobacterium butyricum; paw volume was measured as an indication of inflammation.	Female Wistar rats, 10-16 rats in each group; groups included control, Avemar alone, drug alone, and Avemar + drug.	2 equal daily doses totaling 0, 0.1, 0.5, 2.0, 2.5, 4.0 or 5.0 g/kg/d	15, 21, or 24 days	No toxic effects were observed.

Table A1.2. Human Clinical Trials: Findings Regarding Safety						
Citation	**Objective**	**Study Design**	**Subjects**	**Dose**	**Duration**	**Safety Results**
Ajkay et al. 2000 (unpubl; abstract only)	Assess the effect of Avemar on patients' quality of life in terminal lung cancer.	Open-label uncontrolled cohort trial.	17 terminal lung cancer patients; 5 receiving chemo- and radiotherapy; 9 receiving chemotherapy alone; 3 receiving only palliative therapy.	Not reported.	7.9 mo avg.	No adverse effects were reported; patients had less fatigue and constipation than from therapy alone.
Balogh et al. 2001a (unpubl; abstract only)	Assess the supportive value of Avemar as a postsurgical adjuvant to chemotherapy in the treatment of breast cancer.	Open-label uncontrolled cohort trial.	39 postsurgical breast cancer patients at UICC staging I (n=3), II (n=14), III (n=9), and IV (n=13).	Not reported.	19.3 mo avg.	No adverse effects were reported; patients had fewer side effects than from chemotherapy alone.

Table A1.2. Human Clinical Trials: Findings Regarding Safety						
Citation	**Objective**	**Study Design**	**Subjects**	**Dose**	**Duration**	**Safety Results**
Balogh et al. 2001b (unpubl; abstract only)	Assess the supportive value of long-term use of Avemar as a postsurgical adjuvant in the treatment of breast cancer.	Open-label uncontrolled cohort trial.	55 postsurgical breast cancer patients at UICC staging I (n=8), II (n=19), III (n=15), and IV (n=13).	Not reported.	32.2 mo avg.	No adverse effects were reported; patients had improved physical function; less fatigue, nausea, insomnia, and constipation.
Demidov et al. 2002 (unpubl)	Assess the effect of Avemar + dacarbazine v. dacarbazine alone in postsurgical treatment of skin melanoma patients.	Open-label randomized controlled study with high-risk stage III malignant skin melanoma patients.	22 patients (13 male, 9 female) in test group, 24 (15 male, 9 female) as controls; ages 17-73 years.	9 g/d in a single dose	12 mo	Fewer reports of all adverse events in test than control group: severe nausea, fatigue, fever or infection, leukopaenia, thrombocytopaenia, diarrhea.

Table A1.2. Human Clinical Trials: Findings Regarding Safety						
Citation	**Objective**	**Study Design**	**Subjects**	**Dose**	**Duration**	**Safety Results**
Garami et al. (unpubl and undated)	Assess whether Avemar given with cytotoxic drugs reduces treatment-related sepsis in pediatric cancer patients v. treatment with drugs alone.	Open-label, matched pair controlled pilot clinical trial.	11 randomly chosen matched pairs of children under 18 years (average = 11 years) with proven solid malignant tumors.	12 $g/m^2/d$, in 2 doses; estimated 6.25 g/d, estimated 192 mg/kg/d	29 mo. avg.	No serious adverse events (NCI-CTC Grade 3-4) were observed.
Jakab et al. 2003	Compare antimetastatic activity of Avemar + standard cancer therapy v. standard cancer therapy alone in postsurgical treatment of colorectal cancers.	Open-label controlled multicenter cohort trial; patients chose cohort allocation.	170 colorectal cancer patients from 3 oncosurgical centers; 66 received Avemar, 104 controls	9 g/d in a single dose	18.3 mo avg.	No serious adverse events (NCI-CTC Grades 3-4) were observed. Diarrhea occurred in 4 test-group patients, nausea in 2, and flatulence, repletion, soft stools, and constipation each occurred in 1 case

Table A1.2. Human Clinical Trials: Findings Regarding Safety						
Citation	**Objective**	**Study Design**	**Subjects**	**Dose**	**Duration**	**Safety Results**
Telekes et al. (unpubl and undated)	Perform a pilot study to determine the feasibility of a clinical trial of Avemar in the treatment of rheumatoid arthritis.	Open-label nonrandomized Uncontrolled pilot study.	15 patients with diagnosed rheumatoid arthritis, age 44-68 years, receiving drug therapy.	18 g/d in 2 doses of 9 g each	12 mo	Side effects were evaluated at baseline, 6 months, and 12 months. No adverse side effects of Avemar were observed; no patients withdrew from the study. No change in hemoglobin, liver function, kidney function.
Ujpal et al. (unpubl and undated)	Assess the supportive value of Avemar as a postsurgical adjuvant in advanced squamous cell carcinoma of the oral cavity.	Open-label, comparative, non-randomized single-center trial with historical controls.	22 post-surgical oral cancer patients received Avemar along with standard therapies; 23 patients were historical controls.	9 g/d in a single dose	12 mo	No adverse effects were reported.

APPENDIX 2

Certifications of Quality Control Systems of Biropharma First Hungarian Biotechnological Ltd

APPENDIX 3

Copies of Cited Studies